

- 2.6 P.M. Whole region of solar plexus painted with nicotin sulph.
2.10 „ Stim. splanchnic for 45 sec. Blood pressure remained at same level,
60 mm. Hg. In the tracing there were no respiratory variations,
but at intervals of 25 to 30 sec. there was a slight fall of the blood
pressure.

IV. "On the Tubercles on the Roots of Leguminous Plants, with special reference to the Pea and the Bean." By H. MARSHALL WARD, M.A., F.R.S., F.L.S., late Fellow of Christ's College, Cambridge, Professor of Botany in the Forestry School, Royal Indian Engineering College, Cooper's Hill. Received October 22, 1889.

(Preliminary Paper.)

In the 'Philosophical Transactions' for 1887 (vol. 178, B, pp. 539—562, Pl. 32 and 33) I published the results of some investigations into the structure and nature of the tubercular swellings on the roots of *Vicia faba*, the broad-bean of our gardens, paying attention to the bearing of the facts on other Leguminous plants, and discussing what had been done and written at various times concerning these curious structures.

The chief facts established in that paper were as follows:—That the tubercles occur in all places and at all times on the roots of Papilionaceous plants growing in the open land, but that in sterilised media and in properly conducted water-cultures they are not developed, unless the root is previously infected by contact with the contents of other tubercles. In other words, the tubercles can be produced at will by artificial infection. I also showed that the act of infection is a perfectly definite one, and is due to the entrance into the root-hair of a hypha-like infecting tube or filament, which starts from a mere brilliant dot at the side or apex of the root-hair, passes down the cavity of the latter, traverses the cortex of the root from cell to cell, until its tip reaches the innermost cells of the cortex, where it branches and stimulates these cells to divide and form the young tubercle.

It should be noted that these fruits of the infection were entirely new, as were the methods, and that I showed actual preparations of the infecting filaments passing down the root-hairs, to several botanists at the time (June, 1887).

In my paper were also explanations of several points hitherto obscure—such as the curious trumpet-shaped enlargements of the filaments where they transverse the cell-walls of the tissues, suggesting that they were due to subsequent stretching of the walls of the meristematic cells. Also the peculiar haustorium-like swellings of

intra-cellular filaments, and the minute "bacteroids" (which I termed "gemmules") were described. Again, I called attention to the remarkable coiling and distortion of the root-hairs at the point of origin and entry of the infecting filament. It may be recalled to mind that I wrote of the minute brilliant dot at this spot "unless this dot is one of the above-named 'germs' (*i.e.*, a 'bacteroid' or 'gemmule') I do not know what it can be" (p. 548). I also distinctly pointed out that the twisting of the root-hair at the point of infection might be due to the wall of the root-hair growing elsewhere, but not at that point. As to the "gemmules" or "bacteroids"* with which the cells of the inner parts of the tubercle are filled, and their relations to the filaments, I expressed myself (somewhat cautiously it is true) to the following effect. From their curious shapes—those of the letters V, Y, and X, and even more branched figures—I suggested that these bodies propagate by budding; and from their relations to the swollen ends of the intra-cellular branches of the filaments it was not improbable that they are budded off from these, and multiply by further budding in the protoplasm, &c., of the cells. Owing to the extreme minuteness and high refractive index of these bodies, however, I could not definitely decide as to the method of propagation; although no doubt existed that they are living "germs," on the one hand, and that they originate from the filaments, on the other. I also pointed out that their presence in the protoplasm of the cell stimulates the latter and makes it resemble a plasmodium (p. 547). Other points of importance will be recalled as we proceed.

I have now to draw attention to some results of my further researches into this confessedly difficult subject.

After numerous culture experiments and observations made last year (1888), I have decided to abandon the broad-bean as the subject for histological analysis, chiefly because it takes so long to exhaust its stores of reserve materials; it was better for the cultures to be made with the pea, the cotyledons of which are so much smaller, and the plant of which is more easily managed in every way in water- and pot-cultures. On the other hand, the tubercles and their contents present no essential features of difference, and, indeed, I may say at the outset, that all that has been described with respect to the tubercles of the one is essentially true for those of the other. The position on the roots and the sizes and shapes of the tubercles are the same, and they appear under the same conditions. The colour and general structure of their internal tissues are similar, and the bacteroids of the pea are so little different from those of the bean that it is difficult to believe them specifically distinct.

But I can offer more conclusive evidence than the above for the

* Tschirch's word "bacteroids" is a very convenient one, as it does not commit us to any statement as to the nature of these bodies.

identity of the bacteroids in the two cases. In some of the cultures made in the summer of 1888 I infected the roots of the pea with bacteroids taken from the tubercles of the bean, and as this is a point of some importance, in view of the belief that each species of Leguminosæ may have its own species of bacteroid, I may say a few words on this phenomenon.

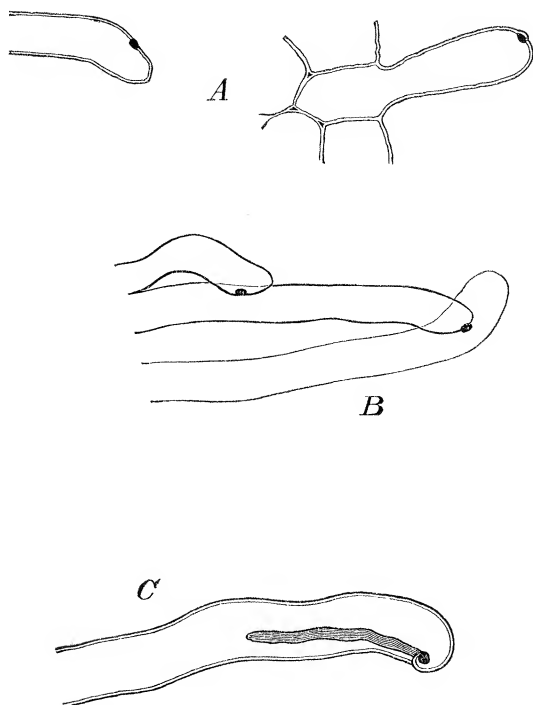
Having satisfied myself that when the contents of the tubercles of the bean are placed among the root-hairs of the young bean root, the latter become infected, I prepared a number of beans and peas as follows: They were allowed to germinate in the sterilised sand until the radicle was about half an inch long; each seedling was then pinned to a cork, and so fixed that the radicle pointed downwards into a large wide-mouthed bottle, in which the cork fitted. The bottle was then carefully lined with filter-paper, kept moist by distilled water; the cork fitted closely, and thus the atmosphere in the bottle was sufficiently damp to enable the radicle to go on growing at the expense of the reserve materials in the cotyledons, and in course of time to put forth a dense pile of delicate root-hairs. As each seedling was pinned on to the cork, I sprayed on to the surface of the radicle, by means of a freshly-drawn capillary tube, a mixture of bacteroids and water made as follows: Tubercles of the bean-roots were carefully washed, placed in alcohol for a few minutes, and then fired, then again washed with distilled water, and pounded in a mortar with distilled water: small drops of this were placed on the radicles as said—*i.e.*, on the radicles of both peas and beans.

In other cases I employed the hanging drops in which I was attempting to cultivate the bacteroids. These consisted of nutritive solutions with asparagin, and with or without gelatine, and in which were placed a few of the bacteroids obtained from cleaned tubercles (cut with a razor sterilised by heat) by means of sterilised needles. I may here say that these cultures (*i.e.*, as micro-cultures) have given me much trouble, and little results: to obtain pure cultures is a matter of greater difficulty than Beyerinck's paper would lead one to expect, and it is not proposed at present to lay much stress on the evidence got from them. Nevertheless, colonies are obtained, and in some cases at least I have transferred the infecting organism from these cultures to the root-hairs of peas and beans.

In any case, I have succeeded in obtaining extracts of the tubercles which contain the infecting germs, and although the latter were always taken from the tubercles of the bean, they infected the root-hairs of both peas and beans equally well.

It is especially the very young root-hairs, with extremely delicate cell-walls, that are infected, and the first sign is the appearance of a very brilliant colourless spot *in* the substance of the cell-wall (figs. A and B): sometimes it is common to two cell-walls of root-hairs in

contact, and not unfrequently one finds several root-hairs all fastened together at the common point of infection (fig. B). This highly refringent spot is obviously the "bright spot" referred to in my pre-



Root-hairs of the pea in process of being infected. A, two very young hairs with the germ in the cellulose wall; B, three root-hairs in the same condition. In C the infecting tube has commenced to grow down the root-hair. The latter is distorted at the point of origin of the tube. The beginning of the distortion is apparent in A (the left-hand figure). A and C = Zeiss J. imm.; B = E obj.

vious paper as the point of infection from which the infecting filament takes origin. It soon grows larger, and develops a long tubular process (fig. C), which grows down inside the root-hair, and invades the cortex, passing across from cell to cell, as described in 1887.

As a matter of fact, then, the "bright spot" is the point of origin of the infecting filament; and, as a matter of inference from the experiments, it cannot but be developed from one of the "bacteroids" or "gemmales" of the tubercles. This attaches itself to the root-hair, fuses with and pierces the delicate cellulose wall, and grows out

into a hypha-like filament at the expense of the cell-contents. The further progress of this filament has already been described in my memoir in the 'Philosophical Transactions' for 1887.

Before proceeding further, reference may be made to some researches made during 1888 and 1889 with the object of learning more about the conditions which rule the development of the tubercles, and the relations of the organism to them. At first I set myself the task of trying to discover a definite spore-stage, thinking that in the rotting tubercles, or at the period of maturity of the plant, or at some other time, it might be that the parasite would betray itself and develop spores: this is not the case apparently, for my experiments seem to prove conclusively that the well-being of the organism of the tubercle and that of the pea or bean go hand in hand. This of course is only so much evidence in favour of the view that we have here a case of symbiosis of the closest kind, as expressed in my previous memoir.

One remark is necessary here. My object throughout had been more especially to determine the nature of the tubercles and of the organism which infests them: the further and larger question as to their function or influence in the economy of the Leguminous plant has been kept subordinate for the present, because I am convinced that more time and appliances are necessary for its complete solution than are at my disposal at present. At the same time some of the following results ought to help in solving the problem as to the possible relations of the tubercle organism to the acquirement of nitrogen by the higher plant.

During the spring and summer of 1888 I made numerous experiments with water-cultures with beans, allowed to germinate in soil so as to be infected by the "germs" therein as demonstrated previously. Several dozens of such cultures were made, and some of them placed in the dark, others in the ordinary light of the laboratory, and some in a well-lighted greenhouse. Tables were prepared showing the number of leaves, living and dead, the condition of the roots, the height of the stem, and so forth, as recorded every week or so (or at shorter intervals) when I examined the plants. It resulted that when the beans are in any way so interfered with that they do not assimilate more material than is necessary for the growth and immediate requirements of the plant, the infecting organism either gains no hold at all on the roots, or it forms only small tubercles which are found to be very poor in "bacteroids:" in some cases the starving plants began to develop tubercles, which never became larger, and in which the infecting organism seemed to be in abeyance. Whether this is due to the bacteroids being developed in small quantities, or to their absorption into the plant is still a question.

I hardened many of these tubercles in picric acid, stained them,

and cut sections, comparing the results with similarly prepared normal tubercles: the chief difference was the paucity in bacteroids, and the prominence of the branched filaments in the cells. Similar results were obtained by placing a box over beans growing in the garden, and comparing the tubercles of the etiolated plants with those of normal plants beside them.

In the spring of this year (1889) I started a series of water-cultures of beans, infected artificially by placing the contents of tubercles on their root-hairs, and kept the roots oxygenated by passing a stream of air through the culture liquid for 24 hours at intervals of a few days: here again the increased growth of the plants—not compensated by increased assimilation—seemed to cause the suppression of the tubercles, or the formation of very poor ones only. These experiments, carried out on several dozens of plants, lead me to conclude that the organism which induces the development of the tubercles is so closely adapted to its conditions that comparatively slight disturbances of the conditions of symbiosis affect its well-being: it is so dependent on the roots of the Leguminosæ, that anything which affects their well-being affects it also.

Some experiments with peas, which are now being tabulated, may throw some light on the wider question which has been raised of late, as to the alleged connection between the development of these tubercles and the increase of nitrogen in Leguminous plants. Thirty-two peas were sown in separate pots of silver-sand, or soil, in five batches of six each, and one of two, and treated in various ways. Six were in garden soil; six in silver-sand, with culture salts, including a nitrate; six in the same medium without nitrate; six in the sand, with traces of soil washings or with pieces of tubercles added; six in sand sterilised by heating; and two in sterilised sand, to which salts (including nitrogen) were added. All but those in the thoroughly sterilised medium bore crops; and these crops have been analysed for me by Professor Green. The soils, water, and other parts are being analysed by Dr. Matthews of Cooper's Hill. I have to thank these gentlemen for the great care and trouble they have kindly taken in this matter.

My object was to decide, if possible, certain points as to the effects of such treatment on the development of the tubercles; but the experiment may possibly turn out more instructive than was at first thought, and will at any rate suggest a line of inquiry to be followed out in the coming spring and summer.

With respect to these plants, I may say that I shall have data showing how much nitrogen was present in the medium at the beginning of the experiment; how much was added; how much the average seedling pea contained; and how much the crop contained in each case. The condition of each plant at convenient intervals was

recorded; the number of living leaves and of dead ones; the height of the stem; number of buds, flowers, and fruits, open and set; and the number of seeds ripened.

The tubercles were developed on all but one of the plants, except those in the completely sterilised media. However, I do not propose to go further into these matters at present, simply contenting myself with pointing out that all the evidence at present goes to show that *the Leguminous plant gains nitrogen by absorbing the nitrogenous substance of the bacteroids from the tubercles*; that nitrogenous substances are thus brought by the "bacteroids" ("gemmules") of the infecting organism of the plant; and that, finally, no satisfactory explanation seems forthcoming as to how the organism obtains this nitrogen in certain cases where no compounds of nitrogen have been added. At any rate, if we regard the pot of sand and its pea as one system, there is in some cases *a distinct gain of nitrogen in the crop, and in the sand at its roots.*

Since the publication of my paper in 1887, several observers have attacked the subject from various points of view. The most important papers are those of Vuillemin,* Beyerinck,† and Prażmowski,‡ who deal with the histology and biology of the subject; and those of Hellriegel and Wilfarth, and of Lawes and Gilbert,§ who have concerned themselves especially with the nitrogen question; papers on accessory matters by Frank, Van Tieghem, and a few others have also appeared in the interval. I propose to deal at present only with Prażmowski's papers, since there are several points in them that have special reference to my work on this subject.

In the 'Botanisches Centralblatt' for 1888,|| appeared a paper by Prażmowski, on the "Root-tubercles of the Leguminosæ." After shortly summarising the literature, and the various views promulgated by different investigators as to the nature of the swellings, the author proceeds to give an account of his own researches. He gives me the credit of having taken a new departure; speaking critically of the want of experimental proofs for their speculative views on the part of previous observers, he says, "*Eine rühmliche Ausnahme bildet in dieser Beziehung Marshall Ward, welcher zuerst in den Weg des physiologischen Experimentes betreten hat*" (p. 217), though he does not regard my methods as complete.

* "Les Tubercules Radicaux des Légumineuses," 'Annales de la Science Agricole française et étrangère,' vol. 1, 1888.

† 'Botanische Zeitung,' 1888, No. 46, p. 725.

‡ 'Botanisches Centralblatt,' 1888, No. 46, pp. 215—285; and 1889, No. 33, p. 356.

§ 'Phil. Trans.,' vol. 180, 1889, B, pp. 1—107.

|| No. 46, pp. 215—285. The paper is an abstract of an address to the Polish Naturalists' Congress, July, 1888.

Experiments with peas and kidney beans enabled him to confirm decisively the discovery that plants in sterilised media, watered with boiled water, develop no tubercles on their roots, whereas those in ordinary soil, or in sterilised media to which infective matter from open soil or from tubercles was added, always formed them. In other words, the tubercles arise by infection from without, as I had demonstrated. He then proved that very young tubercles still show the infecting filaments passing down the root-hairs, "*gewöhnlichen Pilzhypphen nicht unähnliche Fäden, welche, Wurzelhaare und Epidermis durchwachsend, in das subepidermale Gewebe der Wurzel eindringen. Diese Fäden hat schon Marshall Ward in den Wurzelhaaren der Bohne (Vicia faba) beobachtet und auf Grund dieser Beobachtung behauptet, dass die fraglichen Knöllchenorganismen durch Wurzelhaare in die Wurzel eindringen.*" He describes the appearance of the filaments, their bright look, apparent want of membrane at first, granular contents, &c., all in accordance with my statements in 1887.

The granular contents gave him much concern; they are seen as minute rodlets under certain reactions. The branching, piercing of cell-walls, &c., are described as by myself. The only difference here is that Prazmowski believes the rodlets to be the same as the bacteroids.

He completely confirms my observation that the tubercle arises from disturbances produced in the deeper cortical tissues by the infecting filament, and describes the cell-contents, nucleus, &c., so well known. He also points out that in the very young conditions, the bacteroids are simple rodlets, even in cases where they become V, Y, X, &c., shaped later.

To sum up, Prazmowski's account of the whole matter confirms that which I gave to the Royal Society in 1887, excepting that he interprets the origin and nature of the bacteroids differently; he regards them as produced from the *contents* of the filaments—as germ-like bodies developed in the interior of the filaments, and not budded off from them. This is hypothesis only, however, for the author expressly states (p. 253), "*Direct habe ich ihre Theilungen nicht gesehen, obgleich ich mir die Mühe gab, sie in den verschiedensten Nährmedien und unter den verschiedensten äusseren Bedingungen zu züchten.*" He concludes they can only multiply in the still *living* protoplasm.

As to the shapes of the bacteroids and tubercles, Prazmowski's statements agree with those of previous observers, and he also remarks the plasmodium-like appearance of the cell protoplasm at certain stages, as noticed by myself. Some observations on a possible spore formation need not be dwelt upon, as he recognised his mistake in a subsequent paper in 1889.

He leaves the question as to the origin of the bacteroids by budding or otherwise quite undecided, having failed to satisfy himself whether

my suggestion is right or not; at the same time he fully agrees with me and others in believing that these tiny bodies must be the infecting agents, easily and abundantly distributed as they are in the soil, water, &c.

In the 'Botanisches Centralblatt,' vol. 39, No. 12, 1889, p. 356,* is a second paper by Prażmowski, on the nature and biological significance of the root tubercles of the pea, in which he sums up his views so far. He says, the root tubercles of the Papilionaceæ are not normal structures, but are caused by a special fungus, which inhabits the tubercles, and the spores of which must also occur in the soil. Hitherto he had been unable to determine the true nature of this fungus, but only to show that it penetrates through the root-hairs into the young root, grows in it in the form of more or less branched, unseptate tubes, which are clothed by a dense refringent membrane and contain innumerable extremely minute rod-like bodies. Under the influence of this fungus the young tubercle is developed in the deeper parts of the cortex, and in its tissues the bacterium-like contents of the fungus become distributed, and grow, divide, and branch at the expense of the protoplasmic contents. He regarded the phenomenon as one of symbiosis, and as benefiting the host as well as the parasite.

Prażmowski then refers to the papers by Vuillemin, Beyerinck, Hellriegel, and Wilfarth, and says that these instigated him to take up the matter again, and to confine his attention to the pea. The summary of his new results runs as follows:—

The tubercles are not formed in sterilised media unless infected.

The infecting organisms are bacteria, identical in form and properties with those cultivated by Beyerinck from the tubercles of various species. From young tubercles the bacteria can be obtained and cultivated pure, and infections from the cultures cause the tubercles to develop.

The development of the tubercles is only possible in young roots or rootlets; infection does not occur in older portions of the root system.

The tubercle-bacteria penetrate through young (not suberised) cell membranes into the root-hairs and epidermis cells of the root, and there multiply at the expense of the protoplasmic cell-contents. Their further development has so far been observed only in root-hairs. After accumulating and multiplying in the root-hair, they unite in racemose colonies at or near its apex; these colonies become denser and closer, surround themselves with a resistant bright membrane, and join by its means the cell-membrane of the root-hair. Thus arises at the apex of a hair, and on its inner side, a bright knob, usually surrounded by free colonies of bacteria—*i.e.*, colonies not

* This paper is quoted from the 'Berichte a. d. Sitzungen der K. K. Akad. d. Wiss. in Krakau,' June, 1889, and it appears in several journals in the same form.

enveloped by membrane. The top of the root-hair coils round the knob, and the latter then puts forth a tube filled with bacteria and surrounded by a brilliant membrane.

From this stage onwards, till the tubercle is developed, this tube behaves like a hypha, growing at the apex, and putting out branches beneath the apex which behave like the original filament.

The tube now grows from the root-hair, through the cortex, and even as far as the endodermis, boring through the walls of the cortex cells, splitting them mostly into two lamellæ, so that a swelling full of bacteria and bounded by lamellæ is formed. In the cells the tube grows towards the nucleus, and usually applies itself so close that the latter is indistinguishable unless stained. Hence, probably, the reason why Beyerinck regarded the tube as "*schleim*" *débris* remaining over from nuclear division. So far, we have no free bacteria in cells; they are all in the tube.

As soon as the tube reaches the deeper layers of the cortex the cells begin to divide, at first slowly and irregularly, then quickly; and this is especially true of the four or five innermost layers of the cortex. Then also numerous thin branches are developed from the tubes, enter the new cells, and branch in them. The result is the meristem of the tubercle. The middle of the tubercle consists of a parenchyma of larger cells, penetrated by tubes in all planes, and filled later with bacteria freed from the dissolving tubes; the outer layers consist of smaller and more flattened cells with poorer contents, the membranes of which are suberised later. Between the bacteroid tissue and the latter (cortex of tubercle) is a small-celled meristem, free of bacteroids; this produces vascular bundles further back, and these fork and are joined to those of the root. Between the vascular bundles and the bacteroid tissue is further a layer of starch-bearing cells, free of bacteria.

The place where the tubercle forms is predetermined by the infecting tube, and since this enters anywhere, the tubercles arise irregularly, *i.e.*, opposite or not to xylem or phloëm. Pericambium has nothing to do with it, and so Van Tieghem, Beyerinck, and others are wrong in regarding these tubercles as modified lateral roots.

After the tissues of the tubercle are differentiated and the bacteria are set free (parts of the tubes do not burst and dissolve), the latter fuse with protoplasm, multiply by growth and fission, become forked, and subsequently form the bacteroids. The further fate of these depends on their *rôle* in the economy of the plant.

As did Hellriegel, so also Prazmowski put some plants in soils with, others in soils without, nitrogen, and he confirms Hellriegel's results—infected plants require no nitrogen at their roots; non-infected plants pass into a state of hunger and die if not supplied with nitrates.

No decision is arrived at as to whether the nitrogen is got from nitrogen compounds or from the free nitrogen of the air, nor as to what advantage accrues to the bacteria and the host-plant respectively.

As regards the plants' mode of utilising the presence of the bacteria, cultivated bacteria (from pea tubercles) in nutritive media divide indefinitely, and are found there as moving rodlets. In tubercles they are only rodlets while enclosed in tubes; they change their forms in the substance of the protoplasm, becoming forked and developing into bacteroids. As bacteroids they can long go on multiplying by continually developing lateral branches, even in proper nutritive solution (*"In diesem Zustande der Bakteroiden können sie sich noch eine Zeit lang vermehren unter fortwährender Bildung von Seitenzweigen selbst dann, wenn sie aus dem Knöllchen heraus, in geeignete Nährlösung versetzt werden"*). With the further development of the tubercle they become hyaline, cease to multiply, and at length dissolve. The contents of the bacteroid cells are resorbed as the bacteroids dissolve, certain substances being left behind. In other words, the plant utilises the substance of the bacteria.

When emptying begins, and with what energy it proceeds, depend especially on the quantity of nitrogenous compounds at the disposal of the roots. In a soil rich in nitrogen the tubercles go on developing unhindered, become large and typical, and rosy inside, and are not exhausted till late; in poorer soils they attain no great size, are soon emptied, and are green-gray inside.

In both cases the exhaustion proceeds acropetally, from the base onwards. At the apex remains a zone which is not emptied, and its cells are full of bacteria. Moreover, some bacteria in and out of tubes remain in all the cells, and escape during decay into the soil; also animals eat the tubercles and disperse the bacteria. In such injured tubercles the bacteroid masses often envelop themselves anew with membranes, and form smaller and smaller colonies; these the author previously mistook for spores (see p. 438).

From the preceding, we see that the tubercles depend on a symbiosis which is advantageous to both the plant and the bacteria. The latter feed on the sap and cell-contents, and multiply through innumerable generations, and, both during the life of the host and afterwards, become redistributed in the soil. The plant derives advantage in that it obtains nitrogen by means of the bacteria.

Though the symbiosis is useful to both, the plant gains most, for it is the more powerful, and sooner or later overcomes the bacteria, to the multiplication of which it sets limits and finally absorbs the substance of the latter. Being the stronger, the plant directs the symbiosis. It encloses the bacteria in the *"Bakteroidengewebe,"* by means of cork, and also protects them. By an apical meristem the

tubercle provides continual successions of new cells for the bacteria to accumulate in while it absorbs the older ones behind. Between the bacteroid tissue and the cork it provides vascular bundles (1) to feed the bacteria and convey the carbohydrates necessary to produce proteids, and (2) to take up the dissolved substance of the bacteroids as required. The thin cell walls of the bacteroid tissue conduce to the same end.

I think it will be admitted by all who study the literature of this subject, that the only real point at issue between Prazmowski and myself is the nature of the bacteroids and their origin from the filaments. I interpreted them as extremely minute budding "gemmules," and not bacteria; Prazmowski, with Beyerinck, regards them as true Schizomycetes. We have all alike failed to actually see the process of budding or fission, a fact which will surprise no one who has examined these extremely minute bodies, which are, as Beyerinck rightly puts it, among the smallest of living beings.

The fact of infection, and the mode of infection, by means of a hypha-like filament passing down the root-hair were definitely established by myself in 1887, and it is satisfactory to find it confirmed in every essential detail by Prazmowski. Our views as to the symbiosis, the struggle between the protoplasm and the "gemmules" (or "bacteroids") are the same: though Prazmowski and Beyerinck carry the matter a step further in definitely inferring the absorption of the conquered bodies of the latter, a point in part supported by some of my experiments.

As to the occurrence, origin, and structure of the tubercles, Prazmowski's account is simply in accordance with my own; and it is interesting to note how many points of detail—the distortions of the root-hairs, the relations of the branching filaments to the nuclei and cell-contents, and those of the incipient tubercle to the end of the filament, for example—are confirmed by him.

There is one point of extreme importance between Beyerinck and Prazmowski on the one hand, however, and myself on the other; they are positive on the subject of the cultivation of the "bacterium" in nutritive media outside the host-plant—or rather the other symbiont—whereas I feel too little confidence in my cultures to assert that the "germ" is definitely isolated and recognised. It is true, I have obtained colonies in the cultures which may be those referred to by these writers, and I may remark that so long ago as 1887 I wrote that certain flocculent clouds in my cultures may be colonies of the organism in question, as I obtain similar clouds of multiplying "germs" on the root-hairs of my water-cultures. Moreover, in some cases I have clear proof that among the colonies in my cultivations the germ in question existed, because I infected peas and beans with them; but it would be going further than the facts

warrant to claim to have definitely isolated and recognised the "germ" by its morphological characters. Still, enough has been done to enable us to say with some confidence that this will yet be done, even if we do not accept that Prazmowski and Beyerinck have already done it.

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